

# Death in the clouds: ranavirus associated mortality in assemblage of cloud forest amphibians in Nicaragua

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**Abstract.** Amphibian diseases are acknowledged as significant contributors to the decline and extinction of amphibian species. The main culprits currently considered are chytridiomycosis and Ranavirus. In Central America, highly endemic and geographical restricted terrestrial species may be at risk from these diseases. We collected 49 *Agalychnis callidryas* larvae, one *Lithobates forrei* and five unidentified larvae on the Nicaraguan Island Ometepe, all deceased, and skin samples were taken. The presence of Ranavirus was determined using PCR. Ranavirus was found involved in 41 of 55 tadpoles. Forty-one *Agalychnis callidryas*, one *Lithobates forrei* and another five unidentified anuran tadpoles.

**Keywords.** Anurans, mortality, Ranavirus, Nicaragua, Ometepe.

Amphibians are declining in all continents where they occur due to several causes like habitat destruction, habitat degradation, pollutants, climate change and diseases (Stuart et al., 2008).

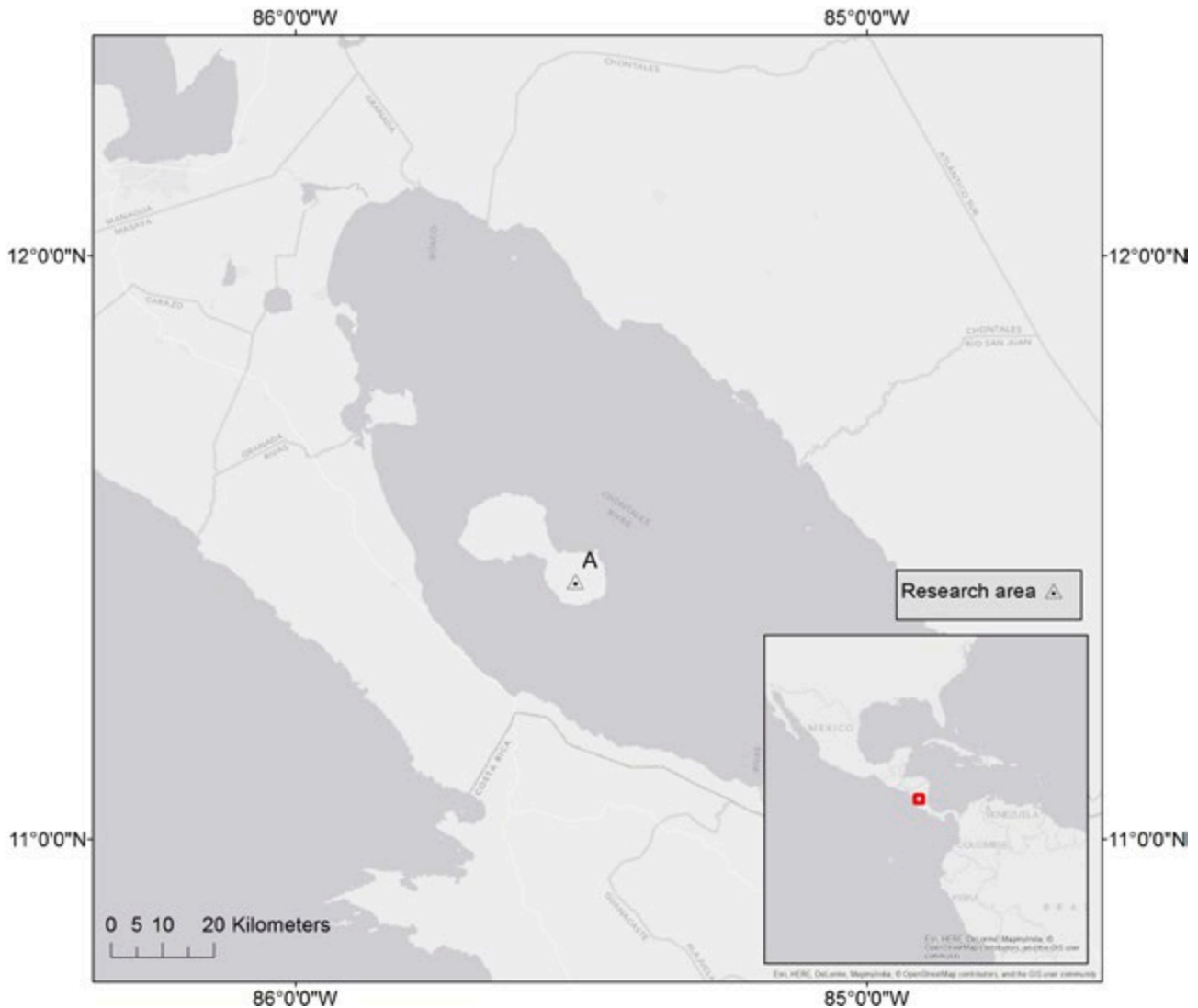
Besides chytridiomycosis caused by the fungal agents *Batrachochytrium dendrobatidis* (Voyles et al., 2009) and *B. salamandrivorans* (Martel et al., 2013), Ranaviruses globally contribute to amphibian declines (Miller et al., 2011; Robert and Chinchir, 2012; Brenes, 2013). Ranaviruses belong to the family Iridoviridae and have been recorded from Asia, Central-, South- and North America and Europe (Whitfield et al., 2007; Xu et al., 2010). Although all amphibian life stages can be affected, larvae appear to be most susceptible to the disease and mortality rates are often high (Green et al., 2002; Greer et al., 2005; Kik et al., 2011; Miller et al., 2011).

Central America is one of the regions considered most struck by disease-driven amphibian declines, notably chytridiomycosis affecting mostly endemic high-ele-

vation species with a restricted range (Duellman, 1999; Savage, 2002; Lips et al., 2008). The volcanic islands in Lake Nicaragua contain complex high-elevation amphibian assemblages in a region where Ranavirus has not been recorded before. In this study we found ranavirus to be involved in a mortality event comprising several anuran species in the crater lake of volcano Maderas on Ometepe Island.

Ometepe (11°30'N, 85°35'W) is situated in Lake Nicaragua (8,264 km<sup>2</sup>) and is comprised out of two volcanos (McKaye, 1995; Nemitz, 2008). Our survey was conducted on the Maderas volcano, the smallest one, with cloud forests on its upper slopes.

This survey was carried out in October 2011, coinciding with the rainy period. Surveys had a strong focus on locating and sampling anurans and their breeding waters. Since breeding animals and vocalizing males were found to be most active during the hours between sunset and midnight, surveys were carried out between these.



**Fig. 1.** Ometepe Island. The study area on Volcano Maderas, one of the two volcanoes that comprises the island, is marked with A.

On 21 October 2011 55 tadpoles of two anuran species (*Agalychnis callidryas* and *Lithobates forrei*), were found dead in the cloud forest of Maderas at an elevation of 1,203 meters (Nicaragua, 11°26'N, 85°30'E). From 54 of these tadpoles, skin swabs were collected and processed for the detection of Bd using qPCR (Boyle et al., 2004) and for the presence of ranavirus DNA using the PCR described by Mao et al. (1997). We tested for these two pathogens because these two are known to cause amphibian declines in Central America. Thirty-five out of 49 dead larvae of *Agalychnis callidryas* (in various stages of development), one dead *Lithobates forrei* tadpole and five dead unidentified tadpoles (due to post mortem decay), tested positive for Ranavirus. None of these larvae tested positive for Bd.

Ranavirus was associated in this case with a mortality event in the larvae of two anuran species on Ometepe. However, only PCR was performed because it was not possible to collect dead animals to perform a histological examination. The latter is needed to confirm an etiological diagnosis (Greer et al., 2005).

During an outbreak, Ranavirus has been known to kill almost 100% of larvae and post metamorphs (Green et al., 2002). Ranavirus may thus have a big impact on geographically restricted and small populations of amphibians on Maderas like the micro-endemic *Bolitoglossa insularis* (status vulnerable IUCN, 2013), *Craugastor laevisissimus* (status endangered IUCN, 2013). As the current study only encompassed a limited time frame, we cannot exclude an effect on other species

within the entire community of amphibians in this cloud forest.

If we consider the most likely scenario that the outbreak is due to a recent introduction of Ranavirus on Ometepe, the route of viral entry is hypothetical. Few tourists venture up Maderas due to the very poor accessibility of the volcano (personal observation). The infrastructure of both the lowland on Ometepe and the accessibility of Maderas are, however, being improved since 2011 (personal observation). With these changes it might be easier to reach the higher ranges of the volcano, which can lead to more people in the cloud forest acting as vectors for Ranavirus. Continuous monitoring of the amphibian assemblages at this site is highly recommended.

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